The relationship between dietary magnesium intake, stroke and its major risk factors, blood pressure and cholesterol, in the EPIC-Norfolk cohort.

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Background: Dietary magnesium could modify the major stroke risk factors, high blood pressure (BP) and cholesterol, but has been understudied in both sexes in a single population. This study aimed to investigate if dietary magnesium intake was associated with BP, total cholesterol (TC) and incident stroke risk in an adult population.

Methods: We conducted cross-sectional analyses in a case-cohort study of 4,443, men and women aged 40-75 representative of 25,639 participants years of the EPIC (European Prospective Investigation into Cancer)-Norfolk cohort. The cohort included 928 stroke cases (42,556.5 person years). Dietary data from 7d food diaries were analysed using multivariate regression to assess associations between quintiles or data-derived categories of dietary magnesium intake and BP, TC and stroke risk, adjusted for relevant confounders.

Results: We observed differences of -7 mmHg systolic BP (P trend ≤0.01) and -3.8 mmHg diastolic BP (P trend 0.01) between extreme intakes of magnesium in men, a significant inverse association with TC was observed (P trend 0.02 men and 0.04 women). Compared to the bottom 10%, the top 30% of magnesium intake was associated with a 41% relative reduction in stroke risk (HR 0.59 95% CI 0.38-0.93) in men.

Conclusions: Lower dietary magnesium intake was associated with higher BP and stroke risk, which may have implications for primary prevention.

1.0 INTRODUCTION

Stroke accounts for more than 5.5 million deaths annually and by 2020 predictions estimate that the global burden of stroke will account for 61 million disability-adjusted life years (1).

Elevated BP[1] is a significant modifiable risk factor for stroke with an approximate fourfold increase in stroke risk in hypertensive individuals compared with the normotensive population (2). Although established evidence indicates that elevated BP, hypertension and circulating cholesterol can be modified by dietary intake including: salt, alcohol, saturated fat and cholesterol (2) other dietary components, including magnesium, which is abundantly available in nuts, green leafy vegetables and whole grains, have been less extensively studied.

Magnesium has a number of metabolic roles in the body and may influence BP and blood lipids through different mechanisms (3, 4). Magnesium may serve as a natural calcium channel blocker, exhibit beneficial effects on platelet coagulation, have a potential role in vasodilation and has been associated with reduced coronary artery calcification (4, 5). Other proposed mechanisms include increased peroxidation of lipoproteins with subsequent acceleration of atherosclerotic plaque formation and low magnesium may facilitate an increase in inflammation which is associated with negative changes in lipid profile (3, 4). Higher magnesium intake has been associated with lower risk of Type II diabetes (6), metabolic syndrome (7) and cardiovascular disease (CVD) (8).

Two recent meta-analyses have investigated the effects of dietary magnesium on stroke risk and CVD risk respectively (8, 9) showing inconsistent findings. The reason for these

[1] Abbreviations
7DD – seven day diet diary, BMI – body mass index, BP – blood pressure, CVD – cardiovascular disease, DBP – diastolic blood pressure, DINER - Data Into Nutrients for Epidemiological Research, EPIC-Norfolk – European Prospective Investigation into Cancer-Norfolk, FFQ – food frequency questionnaire, HDL – high density lipoprotein, HLQ – Health and Lifestyle Questionnaire, LDL – low density lipoprotein, MI – myocardial infarction, SBP – systolic blood press, TC – total cholesterol, WHR – waist-hip ratio

inconsistencies may be due to estimation of magnesium intakes from less precise methods of recording diet such as Food Frequency Questionnaires (FFQ) and 24 hour recalls. However, it has been increasingly suggested that the detailed 7DD represents dietary intakes more precisely (10).

Therefore, the purpose of this study was to determine whether dietary magnesium intake, estimated using a 7DD, was associated with BP, lipid profile and stroke risk in an adult general population of 4,443 (representative of larger cohort 25,639) men and women.

2.0 SUBJECTS AND METHODS

2.1 Study population

The present study population is comprised of a randomly selected representative sample (n=4,000) of the EPIC-Norfolk cohort (n~25,639), which will herein be referred to as EPIC-Norfolk sub-cohort. EPIC-Norfolk has previously been described in detail and the characteristics of the sample was comparable with other representative UK populations with the exception of a lower proportion of current smokers (10). Ethical approval for the study was obtained from the Norwich Ethics Committee, and participants provided informed consent.

Briefly this sub-cohort (n=4,920) is comprised of a representative random sample of 4,000 men and women with complete data for food diaries from the EPIC-Norfolk cohort (n=25,369) and 1,102 stroke cases (n=182 part of 4,000 random sample previously mentioned) giving a total of 4,920. Participants were resident in the Norfolk area at recruitment between 1993 and 1997 and recruited through participating General Practices (n=35) (10). Participants were excluded from analyses if they had reported prevalent stroke at baseline or had missing values for any variables included in the multivariate model (n=477). Participants with missing values for smoking status, aspirin medication use for >3months, and magnesium from supplements

(including medication) were recoded and classified as 'current smoking' (n=37) and 'no' aspirin (n=813) and 'no' supplements (n=2) to reduce the risk of bias due to under-reporting. Therefore 4,443 participants remained for analysis in this study.

2.2 Anthropometric Measures

At baseline participants attended a health check, which took place either at a clinic or the participant's GP surgery, where a number of anthropometric measurements were taken by trained staff according to standardised protocols (10). This included height to the nearest mm, using a free-standing stadiometer. Weight was recorded to the nearest 0.2kg with participants wearing light clothing and no shoes. From this measurement BMI was calculated. Waist and hip measurements were also recorded to the nearest mm (10).

2.3 Clinical and Biological Measures

BP was taken after participants had been seated for 3 mins. Two readings were taken using Accutorr Sphygmomanometer (Datascope, UK) with the participants arm in the horizontal position in line with the mid sternum (10). At the clinic visit, a non-fasting venous blood (42ml) sample was taken from which biochemical analysis for serum cholesterol was conducted (10).

Stroke cases were defined as ICD-9 430-448 or ICD-10 60-69. Fatal and non-fatal stroke incidence was established using death certificate data and linkage with hospital records, using ICD-10 60-69 this method of stroke ascertainment has been shown to have high sensitivity and specificity (11). The current study is based on follow-up to 31st March 2008. Numbers of stroke are given in the tables.

2.4 Lifestyle Factors

Information was obtained from participants on a number of lifestyle variables via a Health and Lifestyle Questionnaire (HLQ). This included smoking status which was categorised as

current, if participants answered “yes” to the question “Do you smoke cigarettes now?”, never if they answered “no” to the question “Have you ever smoked as much as one cigarette a day for as long as a year”. All other participants with valid data were classified as former smokers (missing data were treated as ‘current’). Physical activity was assessed by the use of a short physical activity questionnaire which assessed typical activity over the previous 12 months. Physical activity status took account of both work and leisure related activities and participants were ranked into one of four categories (inactive, moderately inactive, moderately active and active) (10). The repeatability and validity of these was confirmed against heart-rate monitoring (12).

Education level was determined from the HLQ and was defined at the highest qualification obtained at that time. Participants were ranked into one of four categories: ‘degree or equivalent’, A-level or equivalent’, ‘O-level or equivalent’, and less than O-level or equivalent’.

2.5 Previous Medical History

The presence of a number of existing underlying medical conditions was ascertained using the HLQ. Conditions of interest included; stroke, cancer, myocardial infarction and diabetes amongst others. In conjunction with this, participants were requested to detail any medication that they were currently taking.

2.6 Dietary Assessment Method

Participants were requested to record all food and drink items consumed within the 7 day period in a food record diary. Included in each food diary were colour photographs of 17 foods, each with three incremental portion sizes. Participants were requested to indicate which photograph best represented their portion size for each of the items. They were also asked to record the

weight of food items or use household measures to describe the portion size. The use of dietary supplements was also recorded within the diary, and data was input into a specifically designed program ViMiS (vitamin and mineral supplements) (13). The 7-day diary was chosen after validation studies showed its reproducibility and relative validity and indicated that the diet diaries provide a more accurate representation of dietary intakes, over FFQs (10, 14). These studies indicate that FFQs overestimate dietary intakes of a number of food groups including fruit and vegetables, milk and cheese which influence the magnesium intake estimates.

A specific program, DINER (Data Into Nutrients for Epidemiological Research), was developed for entry of dietary information from the 7-day food diaries (15). DINER allows the detailed information provided in diet diaries to be translated into structured data files for nutritional analysis. The program is more flexible than other software which enables the detail of the diary, including cooking method, type of fat used and commercial brand names of products, to be retained (16). Due to the classification structure used to code food items DINER is also able to adapt to changes in food items available on the consumer market (15). The input of items from the food diaries requires a high level of detail, which reduces the risk of bias between coders, and analysis of consistency has echoed this (15). Nutritionists, trained to use the DINERMO program, checked the entered data after which nutrient quantities were calculated and checked for a final time (17).

The ratio of calcium to magnesium intake was calculated by dividing dietary calcium intake by dietary magnesium intake.

2.7 Statistical Methods

All statistical analyses were conducted using the statistical software Stata; version 11 (StataCorp. College Station Tx, 2009). Continuous data are presented as mean with standard error and categorical data as number and percentage. A two-sided P-value of ≤ 0.05 was

considered statistically significant. Independent samples *t test* was used to asses differences in baseline characteristics between men and women.

To account for sex differences associated with a number of variables of interest including BMI and WHR sex specific analyses were conducted.

Multiple regression analysis with multivariate adjustment was employed to assess differences in SBP, DBP and total cholesterol TC with sex specific quintiles of dietary magnesium intake.

Statistical Models

Model 1 comprised of age, BMI, smoking, physical activity (PA) levels, education (all outcomes); use of antihypertensive medications (BP only); baseline reported myocardial infarction (MI) or diabetes, family history of stroke or MI, and use of statin medication (TC only) (2, 18, 19). Model 2, additionally adjusted for dietary factors, including total energy intake in order to demonstrate the effect of magnesium intake independent of total caloric intake, as well as previous incident myocardial infarction (MI) or diabetes at baseline, family history of stroke and MI (BP model only). Alcohol intake, dietary potassium and sodium were included due to their associations with BP (2) and total fat (TC model only). The use of calcium supplements and the ratio of calcium to magnesium intake were included; these two ions antagonise each other, may compete during intestinal absorption and the Ca:Mg ratio may be important for total mortality and coronary heart disease (20).

For stroke risk, model 1 comprised age, BMI, smoking, PA, education and alcohol intake. Model 2 additionally adjusted for serum TC, baseline reported MI or diabetes and family history of stroke or MI. Model 3 included the addition of SBP and DBP, use of aspirin medication >3 months, use of antihypertensive medication, the ratio of dietary Ca:Mg and the use of calcium and magnesium supplements.

A modified Prentice-weighted Cox regression analysis, for case-cohort studies, was used to calculate hazard ratios with 95% CIs for the risk of incident of stroke in association with dietary magnesium intake (21). This modified method accounts for the potential overlap of participants with incident stroke and also randomly present in the representative sub-cohort. Analyses were conducted by sex-stratified data derived categories of magnesium intake with the lowest 10% of intakes (<214mg/d and <180mg/d for men and women), forming the reference category, and subsequent 30% groups of magnesium intake. This approach was taken as we hypothesised that the lowest risk of incident stroke would be in those with the highest dietary magnesium intakes.

Sensitivity analysis was conducted excluding those taking antihypertensive and statin medication respectively.

Total energy was not included as a covariate in cox regression analysis. This was for a number of reasons, including that in the cox regression we adjusted for classical risk factors for stroke and have previously adjusted for total energy in early BP analyses, which indicated that dietary magnesium intake has an effect on BP independently of total energy, specifically for men, and BP was included in cox regression analyses. Additionally with the inclusion of total energy there is potential for collinearity, as a number of covariates included in the model such as BMI, alcohol intake and physical activity are highly correlated with total energy intake. There is also the potential for over adjustment, and for these reasons we chose not to include total energy in the cox regression models.

3.0 RESULTS

In the 4,443 participants included in these analyses 45.0% were male, with an age range of 39-78 years. Mean BP was 140/85 (SD 18.5/11.5) and 136/82 (SD 19.5/11.4) mmHg for males and females respectively (**Table 1**). There was a total of 928 incident strokes during follow-up (mean 9.58 years; total person years 42,556.5) between 1993 and 2008.

Men had significantly higher SBP, DBP and BMI and women had significantly higher TC levels (P for all <0.001), and BMI (P=0.01), but not family history of stroke or MI (P=0.35 and 0.17 respectively), and antihypertensive or lipid lowering medication use (P=0.89 and 0.34 respectively). This illustrates the need to conduct sex-stratified analyses.

Both men and women with the lowest 10% of dietary magnesium intake, compared with the remaining 90% of intakes, tended to be older (64 vs. 61 years, and 63 vs. 60 years for men and women respectively), had a higher percentage of current smokers (18.6% vs. 10.9% and 21.1% vs. 12.0% for men and women respectively), inactive people (42.7% vs. 31.0% and 47.4% vs. 31.2% for men and women respectively) and people taking antihypertensive medication (25.6% vs. 20.3% and 30.2% vs. 20.2% for men and women respectively). There was no substantial difference in BMI, use of statin or aspirin and MI or diabetes at baseline between lowest 10% of magnesium and remaining intakes. Across quintiles of dietary magnesium intake a significantly higher intake of fruit, vegetables and bread and cereals was seen in men and women (P<0.001 for all).

In men but not women, there were inverse associations between dietary magnesium intake and SBP and DBP that remained significant after analysis that accounted for age, dietary sodium intake and use of aspirin or antihypertensive medication (**Table 2**). There were differences of -7 mmHg and -3.8 mmHg between Quintile-1 and Quintile-5 in SBP and DBP (P≤0.01 and

P=0.01 respectively). In women there were no significant associations between dietary magnesium intake and SBP or DBP (Table 2).

Significant inverse associations between dietary magnesium intakes and TC were identified for both genders (P=0.02 in men and P=0.04 in women) after adjustment for anthropometric and lifestyle factors (Table 2). However, these associations were attenuated with the addition of dietary factors; alcohol intake, total fat intake, ratio of Ca:Mg, total energy and calcium supplement intake to the multivariate model but remained significant (P=0.02 in men and P=0.04 in women) (Table 2).

Sensitivity analysis excluding those on antihypertensive medication (n=1583 men and 1927 women) or statin medication (n=1973 men and 2400 women) provided similar results.

Stroke risk showed a non-significant inverse trend across quintiles of dietary magnesium intakes in men and women after adjustment (**Table 3**). In further analyses examining magnesium intake by categories there was a significant trend across categories in men. In those in the highest 30th percentile of dietary magnesium intake (**Table 4**), in men, but not in women (Table 4**)**, there was a 41% relative reduction of stroke risk (HR 0.59 95% CI 0.38-0.93 (P=0.04)) compared to the lowest 10% of magnesium intakes. Although stroke risk was also lower in women this was not significant.

Sensitivity analyses excluding those taking antihypertensive medication attenuated the association of stroke risk in men to be non-significant and strengthened the association in women to be significant and separately excluding those taking statin medication attenuated the association in men to be non-significant.

4.0 DISCUSSION

The main findings of this case-cohort study of British adults suggest that, after adjustment for several important confounding factors including age, smoking status, history of CVD, medication use, total energy intake and other dietary variables, there was a strongly significant association ($P \leq 0.01$) between dietary magnesium intake and SBP and DBP in men, but not in women. There was also an association with TC in both men and women ($P=0.001$ and $P \leq 0.01$ respectively) which was attenuated but remained significant after adjustment for other dietary factors ($P=0.02$ and $P=0.04$ for men and women respectively). Furthermore in relation to stroke risk specifically, we identified a significant decrease in risk (HR 0.59 95% CI 0.38-0.93 $P=0.04$) in men with dietary magnesium intakes ≥354 mg/d (the highest 30%) compared to those with intakes ≤214mg (the lowest 10%).

Compared with our findings, previous studies have shown ~~Previously~~ a significant inverse association between dietary magnesium intake and SBP and DBP in men has also been reported (22) with differences of -6.4 mmHg and -3.1 mmHg for SBP and DBP respectively between those with the highest and lowest intakes in 615 older Japanese men (age 63-82 years) using 24hr recall. Using 7d food diary data, a more robust measure of dietary intakes, in a representative general population of middle and older age we identified slightly greater differences in BP between extreme quintiles of magnesium intakes with a difference of -7 mmHg ($P<0.001$) and -3.8 mmHg ($P<0.001$) for SBP and DBP respectively. We identified between quintiles differences were 250 mg/d for men, and 198mg/d for women, the equivalent to approximately 2 slices of wholemeal bread with peanut butter and 9 Brazil nuts, therefore achievable through dietary intakes (23). We also noted a tendency towards lower fruit, vegetable and bread and cereal intake in those with the lowest dietary magnesium intakes and may be relevant for identifying individuals whom may benefit from increased intake.

In women a potential benefit from increased consumption of magnesium has previously been reported (24-26). Witteman et al (22) and Song et al (23) showed a reduction in relative risk RR 0.77 95%CI: 12%-33% and RR 0.93, 95% CI 0.86-1.02 respectively for developing hypertension in prospective studies using FFQs. A~~lso a~~ meta-analysis of RCTs using oral magnesium supplements also reported a dose dependent effect of supplementation on blood pressure (27). This is in contrast to the current study where we did not find any significant trends between magnesium intake and BP in women. This discrepancy may be due to differences in the models used. For example Witteman et al (1989) (24) did not adjust for lifestyle factors including physical activity levels and smoking status which are known to influence BP. We further explored why we might have identified differences between genders. It may be due to the fact that older age, higher BMI, and higher level of physical inactivity were more prevalent in women with low magnesium intakes which may in part explain why a significant effect was shown in men not women. As highlighted in the results section differences were identified between those with the lowest 10% of intakes and those with higher intakes, however, we took these factors into account during our analyses. It is also of note that differences identified were largely the same for both men and women, although there was a higher percentage of women, with the lowest 10% magnesium intakes, using antihypertensive medication which may influence the findings due to modifying effect of medication on future risk. In addition there was a narrower range of magnesium intakes for women (644mg/d) compared with men (744mg/d) which may attenuate the results. It may also be that the cohort was insufficiently powered to detect an affect in women.

A number of intervention trials, using oral magnesium supplements, have reported significant reductions in BP ranging from 2.0 – 12.0 and 2.7 – 8.0 mmHg for SBP and DBP respectively (28-32). Although, the supplement doses were comparable with dietary intake, ranging between 200 mg/d to 600 mg/d, the formulations used were inconsistent.

Limited studies have previously investigated associations between dietary magnesium intake and TC or subfractions and two previous studies found no association with TC unlike our study which found that TC was ≈4% lower in Quintile-5 compared with Quntile-1 (33-36). However, a higher magnesium intake has been related to beneficial increases in high density lipoprotein (HDL) concentrations (33, 35).

Although several studies have previously investigated stroke risk and dietary magnesium intakes, to our knowledge none have included populations of both men and women simultaneously or included risk factors as well as stroke risk (26, 33-35, 37-42). Previous studies in large populations of American, Taiwanese and Northern European cohorts, which have mainly used food frequency questionnaires (FFQ), have reported no associations (26, 33, 34, 38, 40-42). However, several studies found significant associations in men (35), women (43), and men and women (40). Additionally, a meta-analysis by Larsson et al (2012) (9), in 241,378 people, reported an inverse association between dietary magnesium intake, recorded by FFQ, and risk of stroke. A more recent meta-analysis, of CVD, but not stroke specifically, by Del Gobbo et al (8) in 313,041 participants, concluded that there was no significant association between dietary magnesium intake and CVD. However, a significant inverse association was identified in relation to circulating magnesium and CVD incidence potentially indicating mechanisms that could also affect stroke risk. In addition Guasch-Ferré et al (44) recently reported that an increase in magnesium intake was associated with a decrease in both CVD mortality and total mortality in individuals at high CVD risk. Our results indicated a non-significant trend across quintiles of dietary magnesium intake and stroke risk in men and women (Table 3). However, when we compared men with the lowest 10% of magnesium intake with the remainder of the cohort, we identified a significant inverse trend (P=0.04) across groups in men only (Table 4). This finding would suggest that it is the very lowest magnesium

intakes that may infer the greatest risk of stroke incidence, and the current findings suggest an association between lower dietary magnesium intake and higher stroke risk.

4.1 Strengths and Limitations

The strengths of the present analyses include; the size of the cohort, the representativeness of the UK general population and prospective design for the stroke analyses, which reduces the susceptibility of the study to selection bias. The study design also reduces the likelihood of measurement error, due to the recording of dietary intake at baseline prior to the onset of stroke. Additionally robust and systematic adjustment for a number of potential confounding factors allowed for the identification of dietary associations independent of known risk factors. It is possible that factors not included in the model may also influence associations such as medication use; proton pump inhibitors, and diuretics. The use of quantitative 7-day food diaries is likely to have provided a more accurate representation of micronutrient intakes, including magnesium intake, compared with FFQ and 24-hr recall methods (45). To our knowledge our study is the only one to use dietary intake values from 7-day diaries as opposed to estimates from FFQs. Seven day food diaries have been shown to more accurately represent dietary intakes of a number of food groups that contribute to magnesium intake, including fruit and vegetables, and micronutrient intakes including potassium, carotene and vitamin C in validation studies (14, 45, 46). Despite this, dietary intakes do not account for variation in bioavailability and absorption of magnesium, potentially the use of a biomarker would strengthen the findings (47) and we were also unable to take into account possible contributions of magnesium from drinking water (48). Furthermore in the same population we were able to examine the complex relationship between magnesium intake, BP and cholesterol and stroke risk taking into account potential relevant risk specific confounders.

Selection bias may be possible although the whole EPIC-Norfolk cohort was representative of the UK population, with comparable cohort characteristics, and the sub-cohort for these analyses was representative of the EPIC-Norfolk cohort. Furthermore, truncation of the sample distribution due to potential healthy responder bias would likely only attenuate the observed associations and therefore associations may actually be stronger than are presented. It should also be noted that, as with other cross-sectional and observational longitudinal studies, it is not possible to infer causation from these findings. However, the prospective relationship observed with dietary magnesium intake and stroke risk reduces the likelihood of reverse causality. Furthermore the associations were in agreement with the existing literature. Residual confounding is possible but, the likelihood is reduced due to previous validation of dietary methods and results of the EPIC-Norfolk cohort (46, 49).

4.2 Summary

To our knowledge this is the first study to investigate the association of dietary magnesium with BP, TC and stroke risk in a UK general population of both genders. The results suggest that increased dietary magnesium could positively impact on BP and stroke risk in men and total cholesterol levels in both genders. Our findings suggest that men with the lowest magnesium intakes are at the greatest risk of stroke, lower magnesium intake was also associated with higher blood pressure. Therefore a higher dietary magnesium intake may be beneficial for prevention of stroke in men and warrants further investigation.

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335 Author Contributions

336 Contribution of each author: The research question was formulated by AAW, PKM and LKMB
337 who also analysed the data and wrote the manuscript. KTK and NJW are Principal Investigators
338 of the EPIC-Norfolk. The data collection was organised by AAW, RNL. RNL performed the
339 record linkage. MAHL obtained data from both food and supplement sources using the 7-day
340 diet diaries. All authors contributed to the manuscript and commented on the final version. No
341 authors declare a conflict of interest.

References

1. Mackay J, Mensah GA. The atlas of heart disease and stroke. Geneva: World Health Organization; 2004.
2. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013;31(7):1281-357.
3. Maier JA. Low magnesium and atherosclerosis: an evidence-based link. Molecular aspects of medicine. 2003;24(1-3):137-46.
4. Houston M. The role of magnesium in hypertension and cardiovascular disease. Journal of clinical hypertension. 2011;13(11):843-7.
5. Hruby A, O'Donnell CJ, Jacques PF, Meigs JB, Hoffmann U, McKeown NM. Magnesium intake is inversely associated with coronary artery calcification: the Framingham Heart Study. JACC Cardiovascular imaging. 2014;7(1):59-69.
6. Lopez-Ridaura R, Willett WC, Rimm EB, Liu S, Stampfer MJ, Manson JE, et al. Magnesium Intake and Risk of Type 2 Diabetes in Men and Women. Diabetes care. 2004;27(1):134-40.
7. He K, Liu K, Daviglus ML, Morris SJ, Loria CM, Van Horn L, et al. Magnesium Intake and Incidence of Metabolic Syndrome Among Young Adults. Circulation. 2006;113(13):1675-82.
8. Del Gobbo LC, Imamura F, Wu JH, de Oliveira Otto MC, Chiuve SE, Mozaffarian D. Circulating and dietary magnesium and risk of cardiovascular disease: a systematic review and meta-analysis of prospective studies. The American journal of clinical nutrition. 2013;98(1):160-73.
9. Larsson SC, Orsini N, Wolk A. Dietary magnesium intake and risk of stroke: a meta-analysis of prospective studies. Am J Clin Nutr. 2012;95(2):362-6.
10. Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A, et al. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. British journal of cancer. 1999;80 Suppl 1:95-103.
11. Sinha S, Myint PK, Luben RN, Khaw KT. Accuracy of death certification and hospital record linkage for identification of incident stroke. BMC medical research methodology. 2008;8:74.
12. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public health nutrition. 2003;6(4):407-13.
13. Lentjes MA, Bhaniani A, Mulligan AA, Khaw KT, Welch AA. Developing a database of vitamin and mineral supplements (ViMiS) for the Norfolk arm of the European Prospective Investigation into Cancer (EPIC-Norfolk). Public health nutrition. 2011;14(3):459-71.
14. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public health nutrition. 2002;5(6B):1113-24.
15. Welch AA, McTaggart A, Mulligan AA, Luben R, Walker N, Khaw KT, et al. DINER (Data Into Nutrients for Epidemiological Research) - a new data-entry program for nutritional analysis in the EPIC-Norfolk cohort and the 7-day diary method. Public health nutrition. 2001;4(6):1253-65.

16. Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R, et al. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. Public health nutrition. 2001;4(3):847-58.
17. Lentjes MA, McTaggart A, Mulligan AA, Powell NA, Parry-Smith D, Luben RN, et al. Dietary intake measurement using 7 d diet diaries in British men and women in the European Prospective Investigation into Cancer-Norfolk study: a focus on methodological issues. The British journal of nutrition. 2013:1-11.
18. Myint PK, Sinha S, Luben RN, Bingham SA, Wareham NJ, Khaw KT. Risk factors for first-ever stroke in the EPIC-Norfolk prospective population-based study. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology. 2008;15(6):663-9.
19. Myint PK, Luben RN, Wareham NJ, Welch AA, Bingham SA, Day NE, et al. Combined work and leisure physical activity and risk of stroke in men and women in the European prospective investigation into Cancer-Norfolk Prospective Population Study. Neuroepidemiology. 2006;27(3):122-9.
20. Dai Q, Shu XO, Deng X, Xiang YB, Li H, Yang G, et al. Modifying effect of calcium/magnesium intake ratio and mortality: a population-based cohort study. BMJ Open. 2013;3(2).
21. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika. 1986;73(1):1-11.
22. Joffres MR, Reed DM, Yano K. Relationship of magnesium intake and other dietary factors to blood pressure: the Honolulu heart study. The American journal of clinical nutrition. 1987;45(2):469-75.
23. McCance RA, Widdowson EM. McCance and Widdowson's The composition of foods. 6th summary ed / compiled by Food Standards Agency and Institute of Food Research. ed. Cambridge: Royal Society of Chemistry; 2002.
24. Witteman JC, Willett WC, Stampfer MJ, Colditz GA, Sacks FM, Speizer FE, et al. A prospective study of nutritional factors and hypertension among US women. Circulation. 1989;80(5):1320-7.
25. Song Y, He K, Levitan EB, Manson JE, Liu S. Effects of oral magnesium supplementation on glycaemic control in Type 2 diabetes: a meta-analysis of randomized double-blind controlled trials. Diabetic medicine : a journal of the British Diabetic Association. 2006;23(10):1050-6.
26. Iso H, Stampfer MJ, Manson JE, Rexrode K, Hennekens CH, Colditz GA, et al. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. Stroke. 1999;30(9):1772-9.
27. Jee SHa, Miller Iii ER, Guallar E, Singh VK, Appel LJ, Klag MJ. The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. American Journal of Hypertension. 2002;15(8):691-6.
28. Dyckner T, Wester PO. Effect of magnesium on blood pressure. British medical journal. 1983;286(6381):1847-9.
29. Witteman JC, Grobbee DE, Derkx FH, Bouillon R, de Bruijn AM, Hofman A. Reduction of blood pressure with oral magnesium supplementation in women with mild to moderate hypertension. Am J Clin Nutr. 1994;60(1):129-35.
30. Sacks FM, Brown LE, Appel L, Borhani NO, Evans D, Whelton P. Combinations of potassium, calcium, and magnesium supplements in hypertension. Hypertension. 1995;26(6 Pt 1):950-6.

31. Borrello G, Mastroroberto P, Curcio F, Chello M, Zofrea S, Mazza ML. The effects of magnesium oxide on mild essential hypertension and quality of life. Current Therapeutic Research-Clinical and Experimental. 1996;57(10):767-74.
32. Kawano Y, Matsuoka H, Takishita S, Omae T. Effects of magnesium supplementation in hypertensive patients: assessment by office, home, and ambulatory blood pressures. Hypertension. 1998;32(2):260-5.
33. Ohira T, Peacock JM, Iso H, Chambless LE, Rosamond WD, Folsom AR. Serum and dietary magnesium and risk of ischemic stroke: the Atherosclerosis Risk in Communities Study. American journal of epidemiology. 2009;169(12):1437-44.
34. Song Y, Manson JE, Cook NR, Albert CM, Buring JE, Liu S. Dietary magnesium intake and risk of cardiovascular disease among women. Am J Cardiol. 2005;96(8):1135-41.
35. Larsson SC, Virtanen MJ, Mars M, Mannisto S, Pietinen P, Albanes D, et al. Magnesium, calcium, potassium, and sodium intakes and risk of stroke in male smokers. Archives of internal medicine. 2008;168(5):459-65.
36. Kim MH, Choi MK. Seven dietary minerals (Ca, P, Mg, Fe, Zn, Cu, and Mn) and their relationship with blood pressure and blood lipids in healthy adults with self-selected diet. Biol Trace Elem Res. 2013;153(1-3):69-75.
37. Ascherio A, Rimm EB, Hernan MA, Giovannucci EL, Kawachi I, Stampfer MJ, et al. Intake of potassium, magnesium, calcium, and fiber and risk of stroke among US men. Circulation. 1998;98(12):1198-204.
38. de Oliveira Otto MC, Alonso A, Lee DH, Delclos GL, Bertoni AG, Jiang R, et al. Dietary intakes of zinc and heme iron from red meat, but not from other sources, are associated with greater risk of metabolic syndrome and cardiovascular disease. The Journal of nutrition. 2012;142(3):526-33.
39. Zhang W, Iso H, Ohira T, Date C, Tamakoshi A. Associations of dietary magnesium intake with mortality from cardiovascular disease: the JACC study. Atherosclerosis. 2012;221(2):587-95.
40. Weng LC, Yeh WT, Bai CH, Chen HJ, Chuang SY, Chang HY, et al. Is ischemic stroke risk related to folate status or other nutrients correlated with folate intake? Stroke. 2008;39(12):3152-8.
41. Larsson SC, Virtamo J, Wolk A. Potassium, calcium, and magnesium intakes and risk of stroke in women. American journal of epidemiology. 2011;174(1):35-43.
42. Kaluza J, Orsini N, Levitan EB, Brzozowska A, Roszkowski W, Wolk A. Dietary calcium and magnesium intake and mortality: a prospective study of men. American journal of epidemiology. 2010;171(7):801-7.
43. Adebamowo SN, Spiegelman D, Willett WC, Rexrode KM. Association between intakes of magnesium, potassium, and calcium and risk of stroke: 2 cohorts of US women and updated meta-analyses. Am J Clin Nutr. 2015.
44. Guasch-Ferre M, Bullo M, Estruch R, Corella D, Martinez-Gonzalez MA, Ros E, et al. Dietary magnesium intake is inversely associated with mortality in adults at high cardiovascular disease risk. J Nutr. 2014;144(1):55-60.
45. Bingham SA. Dietary assessments in the European prospective study of diet and cancer (EPIC). European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation. 1997;6(2):118-24.
46. Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, et al. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. The British journal of nutrition. 1994;72(4):619-43.
47. Fine KD, Santa Ana CA, Porter JL, Fordtran JS. Intestinal absorption of magnesium from food and supplements. Journal of Clinical Investigation. 1991;88(2):396.

48. World Health Organization. Calcium and magnesium in drinking-water: public health significance: World Health Organization; 2009.
49. Bingham SA, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, et al. Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. International journal of epidemiology. 1997;26 Suppl 1:S137-51.

Tables

Table 1. Baseline characteristics by sex in 4443 men and women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997)

	Men	**Women**	**P-value[1]**
	n=2000	**n=2443**	
Age (years)	61.1 (±9.53)	60.4 (±9.71)	0.02
BMI (kg/m$^{2)}$	26.5 (±3.18)	26.2 (±4.24)	<0.01
Family History Stroke (%)	465 (23.3%)	601 (24.6%)	0.29
Family History MI (%)	720 (36.0%)	934 (38.2%)	0.13
Family History DM (%)	222 (11.1%)	305 (12.5%)	0.16
Blood Pressure mmHg			
SBP	140 (±18.5)	136 (±19.5)	<0.001
DBP	85.3 (±11.5)	81.8 (±11.4)	<0.001
PP	54.2 (±11.2)	54.0 (±11.4)	0.66
Antihypertensive Use (%)	417 (20.9%)	516 (21.1%)	0.83

Aspirin Use (%)	271 (13.6%)	197 (8.06%)	<0.001
Blood Lipids mmol/L			
Total Cholesterol	6.07 (±1.10)	6.36 (±1.22)	<0.001
Smoking (%)			
Current	234 (11.7%)	314 (12.9%)	<0.001
Former	1114 (55.7%)	774 (31.7%)	
Never	652 (32.6%)	1355 (55.5%)	
Physical Activity (%)			
Inactive	644 (32.2%)	800 (32.8%)	<0.001
Moderately Inactive	476 (23.8%)	790 (32.3%)	
Moderately Active	440 (22.0%)	514 (21.0%)	
Active	440 (22.0%)	339 (13.9%)	
Education Level (%)			
0 – No Qualifications	667 (33.4%)	1086 (44.5%)	<0.001
1 – O-Level or Equivalent	165 (8.3%)	249 (10.2%)	

2 – A-Level or Equivalent	887 (44.4%)	822 (33.7%)	
3 – Degree or Equivalent	281 (14.1%)	286 (11.7%)	
Dietary Factors			
Total Energy (kcal/d)	2218 (±505)	1685 (±384)	<0.001
Magnesium (mg/d)	318 (±92.0)	265 (±73.2)	<0.001
Ca:Mg Ratio	2.93	2.93	0.96
Potassium (mg/d)	3423 (±819)	2962 (±683)	<0.001
Alcohol (g/d)	15.9 (±20.8)	7.70 (±11.7)	<0.001
Sodium (mg/d)	3150 (±864)	2405 (±660)	<0.001
Calcium Supplement Use (%)	34 (1.70%)	160 (6.55%)	<0.001
Magnesium supplement use (%)	22 (1.10%)	53 (2.17%)	<0.01

[1]P-value difference between males and females.

Values are mean and standard deviations where continuous and number and percentage where categorical.

Table 2. Association of quintiles of dietary magnesium intake (range and mean quintile intake) and blood pressure and total cholesterol (means and SE) in 4443 men and women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

Men		**Dietary Magnesium Intake**					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		85-242 mg	**243-284 mg**	**285-328 mg**	**329-385 mg**	**386-829**	**P for trend**
		206 mg	**266 mg**	**307 mg**	**355 mg**	**456 mg**	
		n=400	**n=400**	**n=400**	**n=400**	**n=400**	
SBP	Unadjusted	143 (±0.98)	140 (±0.97)	140 (±0.88)	139 (±0.90)	136 (±0.87)	<0.001
	Model 1[1]	140 (±0.87)	139 (±0.86)	140 (±0.86)	140 (±0.86)	139 (±0.87)	0.64
	Model 2[2]	143 (±1.16)	140 (±0.90)* [3]	140 (±0.85)*	138 (±0.89)**	136 (±1.18)***	0.002
DBP	Unadjusted	86.1 (±0.58)	85.9 (±0.60)	85.4 (±0.57)	85.2 (±0.56)	84.1 (±0.56)	0.008
	Model 1	85.4 (±0.57)	85.4 (±0.56)	85.3 (±0.56)	85.5 (±0.56)	85.0 (±0.57)	0.68
	Model 2	87.15 (±0.76)	86.1 (±0.59)	85.1 (±0.55)*	84.9 (±0.58)*	83.4 (±0.77)**	0.01

Women							
		48-204 mg	**205-240 mg**	**241-274 mg**	**275-319 mg**	**320-692 mg**	**P for trend**
		176 mg	**223 mg**	**258 mg**	**295 mg**	**374 mg**	
		n=489	**n=489**	**n=489**	**n=489**	**n=488**	
SBP	Unadjusted	140 (±0.90)	135 (±0.85)	137 (±0.93)	135 (±0.89)	133 (±0.81)	<0.001
	Model 1	136 (±0.79)	135 (±0.77)	137 (±0.77)	136 (±0.77)	135 (±0.78)	0.85
	Model 2	137 (±1.07)	135 (±0.82)	137 (±0.77)	135 (±0.81)	135 (±1.09)	0.45
DBP	Unadjusted	83.5 (±0.51)	81.5 (±0.50)	82.4 (±0.55)	80.9 (±0.53)	80.7 (±0.48)	<0.001
	Model 1	82.0 (±0.49)	81.3 (±0.48)	82.6 (±0.48)	81.4 (±0.48)	81.7 (±0.49)	0.71
	Model 2	82.5 (±0.67)	81.6 (±0.51)	82.5 (±0.48)	81.1 (±0.51)	81.2 (±0.68)	0.26
Men							
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	

		85-242 mg	**243-284 mg**	**285-328 mg**	**329-385 mg**	**386-829**	**P for trend**
		206 mg	**266 mg**	**307 mg**	**355 mg**	**456 mg**	
		n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Total Cholesterol	Unadjusted	6.21 (±0.06)	6.16 (±0.06)	6.02 (±0.05)	6.05 (±0.06)	5.91 (±0.05)	<0.001
	Model 1[4]	6.17 (±0.06)	6.16 (±0.06)	6.03 (±0.05)	6.06 (±0.05)	5.93 (±0.06)**	0.001
	Model 2[5]	6.18 (±0.07)	6.16 (±0.06)	6.03 (±0.05)	6.06 (±0.06)	5.94 (±0.07)*	0.02
Women							
		48-204 mg	**205-240 mg**	**241-274 mg**	**275-319 mg**	**320-692 mg**	**P for trend**
		176 mg	**223 mg**	**258 mg**	**295 mg**	**374 mg**	
		n=489	**n=489**	**n=489**	**n=489**	**n=488**	
Total Cholesterol	Unadjusted	6.67 (±0.06)	6.35 (±0.05)	6.28 (±0.05)	6.32 (±0.05)	6.16 (±0.05)	<0.001

Model 1[3]	6.51 (±0.05)	6.33 (±0.05)*	6.31 (±0.05)*	6.36 (±0.05)*	6.26 (±0.05)**	0.005
Model 2[4]	6.52 (±0.06)	6.34 (±0.05)*	6.31 (±0.05)*	6.35 (±0.05)	6.25 (±0.06)*	0.04

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, potassium, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication)

[3]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI or diabetes, family history stroke, family history MI, statin medication use

[4]Model 2: model 1 + alcohol, dietary total fat intake, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication).

[5]P value for significance compared with Q1: *= P value ≤0.05, ** = P value ≤0.005, *** = P value ≤0.001

Table 3. Quintiles of dietary magnesium intake (range and mean quintile intake) at baseline (1993-1997) and stroke risk (HR and 95%CI), follow-up March 2008, in 4443 men and women, aged 40-75 in EPIC-Norfolk cohort.

Men						
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	
	85-242 mg **206 mg** **n=400**	**243-284 mg** **266 mg** **n=400**	**285-328 mg** **307 mg** **n=400**	**329-385 mg** **355 mg** **n=400**	**386-829** **456 mg** **n=400**	**P trend**
Stroke Events	126 (30.6)	111 (26.9)	93 (22.6)	85 (20.6)	75 (18.3)	
Model 1[1]	1.0 (reference)	0.85 (0.60-1.20)	0.70 (0.49-1.00)	0.86 (0.60-1.24)	0.80 (0.55-1.16)	0.22
Model 2[2]	1.0 (reference)	0.86 (0.60-1.20)	0.68 (0.47-0.99)	0.81 (0.56-1.17)	0.74 (0.50-1.09)	0.11
Model 3[3]	1.0 (reference)	0.87 (0.61-1.25)	0.73 (0.50-1.06)	0.80 (0.55-1.17)	0.81 (0.53-1.22)	0.21
Women						

	48-204 mg **176 mg** **n=489**	**205-240 mg** **223 mg** **n=489**	**241-274 mg** **258 mg** **n=489**	**275-319 mg** **295 mg** **n=489**	**320-692 mg** **374 mg** **n=488**	
Stroke Events	152 (30.5)	102 (20.5)	87 (17.5)	82 (16.5)	88 (17.7)	
Model 1[1]	1.0 (reference)	0.74 (0.53-1.05)	0.74 (0.51-1.06)	0.84 (0.59-1.20)	0.83 (0.57-1.20)	0.39
Model 2[2]	1.0 (reference)	0.71 (0.50-1.01)	0.71 (0.49-1.03)	0.82 (0.57-1.17)	0.76 (0.52-1.11)	0.23
Model 3[3]	1.0 (reference)	0.72 (0.50-1.04)	0.73 (0.50-1.08)	0.86 (0.59-1.26)	0.82 (0.54-1.24)	0.45

[1]model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication)

Table 4. Stroke risk (HR 95% CI) by magnesium groups (range and mean intake), bottom 10% (Group 1 reference category) and 3 groups of 30% intakes each, in 4443 men and women, aged 40-75 in EPIC-Norfolk cohort.

Men	**Group 1** **85-214 mg** **181 mg** **n=199**	**Group 2** **215-285 mg** **254 mg** **n=605**	**Group 3** **286-353 mg** **318 mg** **n=591**	**Group 4** **354-828mg** **427 mg** **n=605**	**P trend**
Stroke Events	65 (32.7%)	157 (26.0%)	123 (20.8%)	104 (17.2%)	
Model 1[1]	1.00	0.73 (0.50-1.07)	0.63 (0.43-0.94)*[4]	0.67 (0.44-1.01) *[4]	0.07
Model 2[2]	1.00	0.72 (0.48-1.07)	0.61 (0.41-0.92) *[4]	0.61 (0.40-0.94) *[4]	0.03
Model 3[3]	1.00	0.67 (0.45-1.01)*	0.60 (0.40-0.90) *[4]	0.59 (0.38-0.93) *[4]	0.04

Women	**Group 1**	**Group 2**	**Group 3**	**Group 4**	
	48-180 mg	**181-240 mg**	**241-294 mg**	**295-691 mg**	
	156 mg	**213 mg**	**267 mg**	**352 mg**	
	n=232	**n=745**	**n=740**	**n=726**	
Stroke Events	73 (31.5%)	165 (22.2%)	126 (17.0%)	115 (15.8%)	
Model 1[1]	1.00	0.73 (0.49-1.08)	0.70 (0.46-1.06)	0.74 (0.48-1.12)	0.27
Model 2[2]	1.00	0.67 (0.45-1.00) *[4]	0.65 (0.43-0.98) *[4]	0.66 (0.43-1.02)	0.14
Model 3[3]	1.00	0.65 (0.43-0.99) *[4]	0.65 (0.42-1.01)	0.69 (0.44-1.09)	0.27

[1]model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication)

[4]P value for significance compared with reference (Group 1): *= P value ≤ 0.05